A Theoretical Model for an Immobilized Oxidase Enzyme Electrode in the Presence of Two Oxidants

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Abstract

A theoretical model of an immobilized oxidase enzyme electrode is discussed. The model is based on three reaction/diffusion equations containing a non-linear reaction term under the steady state conditions. An analytical expression pertaining to concentrations of the immobilization of three enzyme substrates are obtained. Furthermore, we employ Homotopy perturbation method (HPM) to solve the system of non-linear reaction/diffusion equations. Simple and an approximate polynomial expression of concentration of substrate, oxygen and oxidized mediator and current were obtained in terms of the Thiele moduli and the small values of parameters B_s (normalized surface concentration of substrate), B_o (normalized surface concentration of oxygen) and B_m (normalized surface concentration of oxidized mediator). The minimum value of the dimensionless concentration of F_s, F_o and F_m in terms of parameter is also reported. Furthermore, in this work the numerical simulation of the problem is also reported using Matlab program. An agreement between analytical expressions and numerical results is observed.

Keywords

Enzyme Electrodes; Non-linear Reaction/Diffusion Equation; Homotopy Perturbation Method; Biosensor

Introduction

The concept of enzyme electrode was first introduced by Clark and Lyons [1] over fifty years ago. Various kinds of biosensors based on electrochemical enzyme electrodes have been studied and a majority of these devices operates in an amperometric mode [2]. Schulmeister [3, 4] described a model for a modified amperometric enzyme electrode with a multilayer enzyme membrane. This model is employed to describe the behavior of the electrode in enzyme kinetics. The kinetics is described by a parabolic differential equation with linear inhomogeneities. Leypoldt and Gough [5] have proposed a mathematical model for glucose oxidase immobilized enzyme electrode for various diffusion coefficients, and kinetic parameters corresponding to an enzymatic reaction. Bergel and Comtat [6] studied the transient response of a mediated amperometric enzyme electrode using an implicit finite difference method. A model for two-substrate enzyme electrode was described by Gooding and Hall [7].

The importance of enzyme electrode and the various approaches to enzyme electrochemistry has been discussed. The current response has been also observed experimentally for mediated enzyme electrodes employing glucose oxidase and other oxidases [8-10]. In recent years homogeneous membranes are widely used as carriers for immobilization of enzymes [11]. They have been utilized in biomaterials, bio-separators and biosensors [12]. The membranes provide an ideal support for the immobilization of the biocatalyst. Substrate partition at the membrane/fluid inter-phase can be used to improve the selectivity of the catalytic reaction towards the desired products [13].

Recently, Martens and Hall [14] have described a model for three-substrate homogeneous enzyme electrode and employ the model to investigate the influence of oxygen on the current response of a mediated oxidase electrode. To our knowledge, no general analytical expressions that describe the concentration of substrate, oxygen and oxidized mediator for various values of the Thiele moduli Φ_s^2, Φ_o^2 and Φ_m^2 and the normalized parameters B_s, B_o and B_m have been reported. However, in general, analytical solutions of non-linear differential equations are more important and very useful than purely numerical solutions, as they are amenable to various kinds of data manipulation and analysis. For

this reason, we have derived that analytical expressions corresponding to the concentrations of substrate, oxygen and oxidized mediator in an oxidase enzyme electrode using Homotopy perturbation method. This method is reliable and highly accurate in handling non-linear problems.

Mathematical Formulation of the Boundary Value Problem

The details of the model adopted have been fully described in Martens and Hall [14] and so we only present a brief summary here. Fig. 1 represents the general kinetic reaction scheme of enzyme-membrane electrode geometry [15].

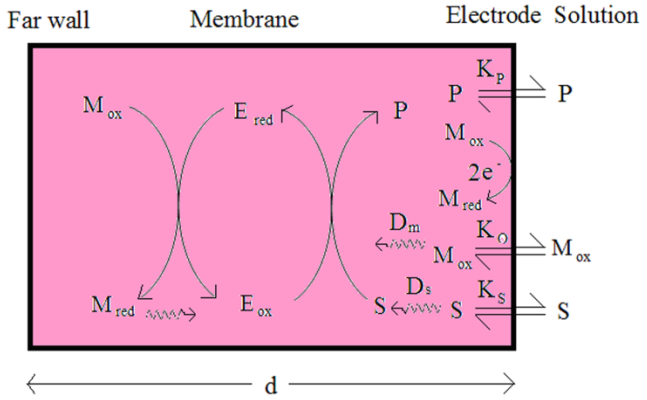

FIG. 1 SCHEMATIC MODEL OF AN ENZYME-MEMBRANE ELECTRODES [12]

Eqs. 1-3 represent the general reaction scheme that occurs often at an electrode immobilized with an enzyme oxidase in the presence of two oxidants:

$$\mathrm{E_{ox} + S \underset{k_2}{\overset{k_1}{\rightleftarrows}} ES \xrightarrow{k_3} E_{red} + P} \qquad (1)$$

$$\mathrm{E_{red} + O_2 \xrightarrow{k_4} E_{ox} + H_2O_2} \qquad (2)$$

$$\mathrm{E_{red} + Med_{ox} \xrightarrow{k_5} E_{ox} + Med_{red}} \qquad (3)$$

We assume that the concentrations of all reactants and enzyme intermediates remain constant for all time. Also the concentration of total active enzyme $[E_t]$ and the reactants in the bulk electrode remain constant. We can consider that the diffusion of the reactants can be described by Fick's second law and the enzymes are assumed to be uniformly dispersed throughout the matrix. The enzyme activity is not a function of position. The reaction/diffusion equations corresponding to the concentrations of substrate, oxidized mediator and oxygen within a matrix can be expressed as [14]

$$D_s \frac{d^2[S]}{dy^2} = k_3[E_t]\left[1+\left(\frac{[O_2]}{\beta_o}+\frac{[Med_{ox}]}{\beta_m}\right)^{-1}+\frac{\beta_s}{[S]}\right]^{-1} \qquad (4a)$$

$$D_m \frac{d^2[Med_{ox}]}{dy^2}\left(\frac{[O_2]/\beta_o+[Med_{ox}]/\beta_m}{[Med_{ox}]/\beta_m}\right) = k_3[E_t]\left[1+\left(\frac{[O_2]}{\beta_o}+\frac{[Med_{ox}]}{\beta_m}\right)^{-1}+\frac{\beta_s}{[S]}\right]^{-1} \qquad (4b)$$

$$D_o \frac{d^2[O_2]}{dy^2}\left(\frac{[O_2]/\beta_o+[Med_{ox}]/\beta_m}{[O_2]/\beta_o}\right) = k_3[E_t]\left[1+\left(\frac{[O_2]}{\beta_o}+\frac{[Med_{ox}]}{\beta_m}\right)^{-1}+\frac{\beta_s}{[S]}\right]^{-1} \qquad (4c)$$

where $[S]$, $[Med_{ox}]$ and $[O_2]$ are the concentrations of substrate, oxidized mediator and oxygen and y is distance from the electrode. D_s, D_m and D_o are the diffusion coefficients of substrate, oxidized mediator and oxygen. $[E_t]$ Denotes the concentration of the total active enzyme in the matrix and $\beta_s = (k_2 + k_3)/k_1$, $\beta_o = k_3/k_4$ & $\beta_m = k_3/k_5$ represent reaction constants of substrate, oxygen and oxidized mediator respectively. The boundary conditions are given by

When $y = d$,

$$[O_2] = [O_2]_b = K_o[O_2]_\infty,\ [S] = [S]_b = K_s[S]_b,\ [Med_{ox}] = [Med_{ox}]_b = K_m[Med_{ox}]_\infty \qquad (5a)$$

when $y = 0$,

$$[Med_{ox}] = [Med_{ox}]_b,\ d[O_2]/dy = d[S]/dy = 0 \qquad (5b)$$

Here $[S]_b$, $[O_2]_b$, $[Med_{ox}]_b$ & $[S]_\infty$, $[O_2]_\infty$, $[Med_{ox}]_\infty$ are the bulk concentrations of substrate, oxygen and oxidized mediator species. K_o, K_s and K_m are the partition coefficients for oxygen, substrate and oxidized mediator respectively. Eq. 4 can be represented in the normalized form using the following normalized parameters.

$$F_s = \frac{[S]}{[S]_b};\ F_o = \frac{[O_2]}{[O_2]_b};\ F_m = \frac{[Med_{ox}]}{[Med_{ox}]_b};\ x = \frac{y}{d};$$

$$B_s = \frac{[S]_b}{\beta_s};\ B_o = \frac{[O_2]_b}{\beta_o};\ B_m = \frac{[Med_{ox}]_b}{\beta_m}; \quad (6)$$

$$\Phi_s^2 = \frac{d^2 k_3 [E_t]}{D_s [S]_b};\ \Phi_o^2 = \frac{d^2 k_3 [E_t]}{D_o [O_2]_b};\ \Phi_m^2 = \frac{d^2 k_3 [E_t]}{D_m [Med_{ox}]_b}$$

where F_s, F_o, and F_m represent the normalized concentrations of substrate, oxygen and oxidized mediator and B_s, B_o, and B_m are the corresponding normalized surface concentrations. The surface concentration is the ratio of the bulk concentration and the reaction constants. Φ_s, Φ_o, and Φ_m denote the Thiele moduli of substrate, oxygen and oxidized mediator, respectively. Thiele modulus Φ^2 represents the ratio of the characteristic time of the enzymatic reaction to that of substrate diffusion. *d* is the thickness of the enzyme layer. The coupled three non-linear reaction/diffusion equations in normalized form are

$$\frac{1}{\Phi_s^2}\frac{d^2 F_s}{dx^2} = \left(1 + \frac{1}{F_o B_o + F_m B_m} + \frac{1}{F_s B_s}\right)^{-1} \quad (7a)$$

$$\frac{1}{\Phi_o^2}\frac{d^2 F_o}{dx^2}\left(\frac{F_o B_o + F_m B_m}{F_o B_o}\right) = \left(1 + \frac{1}{F_o B_o + F_m B_m} + \frac{1}{F_s B_s}\right)^{-1} \quad (7b)$$

$$\frac{1}{\Phi_m^2}\frac{d^2 F_m}{dx^2}\left(\frac{F_o B_o + F_m B_m}{F_m B_m}\right) = \left(1 + \frac{1}{F_o B_o + F_m B_m} + \frac{1}{F_s B_s}\right)^{-1} \quad (7c)$$

The boundary conditions become

$$F_s = F_o = F_m = 1 \text{ at } x = 1 \quad (8a)$$

$$F_m = 1,\ \frac{dF_o}{dx} = \frac{dF_s}{dx} = 0 \text{ at } x = 0 \quad (8b)$$

The normalized current J_{ox} is given by,

$$J_{ox} = \left(\frac{dF_m}{dx}\right)_{x=0} \quad (9)$$

Analytical Expressions of Concentrations of Substrate, Oxygen and Oxidized Mediator under Steady-state Condition

Recently, Ji-Huan He solved many non-linear diffusion equations using Homotopy perturbation method [16, 17]. The Homotopy perturbation method is a reliable and efficient method to solve the non-linear problems [18-24]. The basic principle of this method is described in [21]. Detailed derivations of dimensionless concentration profiles are described in Appendix A. As a result, we have obtained the analytical expression corresponding to the concentrations of substrate, oxygen and oxidized mediator as follows:

$$F_s(x) = \Phi_s^2\left(1 - x^2\right)\left(2 + \frac{2}{B_o + B_m} + \frac{2}{B_s}\right)^{-1} \quad (10)$$

$$F_o(x) = 1 - \frac{\Phi_o^2 B_o\left(1 - x^2\right)}{\left(B_o + B_m\right)}\left(2 + \frac{2}{B_o + B_m} + \frac{2}{B_s}\right)^{-1} \quad (11)$$

$$F_m(x) = 1 - \frac{\Phi_m^2 B_m\left(x - x^2\right)}{\left(B_o + B_m\right)}\left(2 + \frac{2}{B_o + B_m} + \frac{2}{B_s}\right)^{-1} \quad (12)$$

From Eq. 9, we can obtain the current as follows:

$$J_{ox} = \frac{\Phi_m^2 B_m}{\left(B_o + B_m\right)}\left(2 + \frac{2}{B_o + B_m} + \frac{2}{B_s}\right)^{-1} \quad (13)$$

Eqs. 10-12 represent the new analytical expression of concentrations of substrate, oxygen and oxidized mediator.

Numerical Simulation

The three reaction/diffusion equations (Eqs. 7*a*-7*c*) corresponding to the boundary conditions (Eqs. 8*a* and 8b) were solved by numerical methods. We have used pdex4 to solve these equations (Pdex4 in MATLAB is a function to solve the initial-boundary value problems of differential equations). The numerical solution is compared with our analytical results and is shown in Fig. 5. A satisfactory agreement is noticed for various values of the Thiele moduli Φ_s^2, Φ_o^2, and Φ_m^2 and possible small values of parameters B_s, B_o and B_m. Tables 1-3 illustrate the comparison of analytical result obtained in this work with the numerical result. The relative difference between numerical and analytical results is within 3% for all possible values of parameter considered in this work.

Discussion

The kinetic response of a biosensor depends on the concentrations of substrate, oxygen, and mediator. The concentrations of substrate, oxygen and mediator depends on the following four factors Φ^2, B_s, B_o and B_m. The variation in the Thiele modulus can be achieved by varying either the

thickness of the enzyme layer or the amount of enzyme immobilized in the matrix. The Thiele modulus is indicative of the competition between the diffusion and reaction in the enzyme layer. When Φ^2 is small, the kinetics dominate and the uptake of oxygen, mediator and substrate in the enzyme matrix is kinetically controlled. Under these conditions, the substrate concentration profile across the membrane is essentially uniform. The overall kinetics are governed by the total amount of active enzyme $[E_t]$. Diffusion limitations are the principal determining factor when Thiele modulus is large.

A series of normalized concentration profiles for substrate F_s, oxygen F_o, and mediator F_m for various values of diffusion coefficient of substrate D_s, oxygen D_o, and mediator D_m respectively are presented in Fig. 2. From Figs. 2*a* and 2*b*, it is concluded that the concentration profiles of substrate and oxygen increases and reaches the maximum value when the diffusion coefficients of substrate and oxygen increases. The concentration of oxidized mediator starts from the value of unity, goes through a minimum and then increases back to unity as the diffusion coefficient of mediator is increased (Fig. 2*c*).

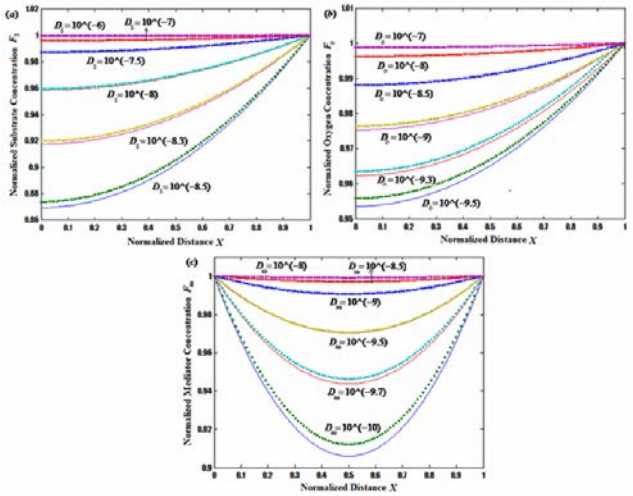

FIG. 2 NORMALIZED CONCENTRATION PROFILES FOR (*a*) THE SUBSTRATE F_s, (*b*) THE OXYGEN F_o, AND (*c*) THE OXIDIZED MEDIATOR F_m. THE CURVES ARE PLOTTED USING EQS. 10 – 12 FOR VARIOUS VALUES OF THE DIFFUSION COEFFICIENTS OF SUBSTRATE, OXYGEN, AND OXIDIZED MEDIATOR AND SOME FIXED VALUES OF PARAMETERS $B_s = 0.5$, $B_o = 0.01$, $B_m = 0.1$, $d = 0.01$ CM, $k_3 = 0.01$ LMOL^{-1}S^{-1}, $[E_t] = 0.005$ MMOL/L, AND $[S]_b = [O_2]_b = [M_{red}]_b = 0.5$ MMOL/L. SOLID LINES REPRESENT THE ANALYTICAL SOLUTION PRESENTED IN THIS WORK AND DOTTED LINES THE NUMERICAL SIMULATION

The normalized concentration profiles of substrate F_s, oxygen F_o, and mediator F_m for various values of the normalized reaction constants $\beta_s = (k_2 + k_3)/k_1$, $\beta_o = k_3/k_4$ & $\beta_m = k_3/k_5$ are shown in Fig. 3. From Fig. 3, all curves increase as the reaction constants increase while keeping the other parameter fixed. From this figure, it is evident that the concentration profiles attains the steady state when the value of reaction constants ≥ 100. Fig. 4 represent the normalized concentration profiles for various values of the Thiele moduli Φ_s^2, Φ_o^2, and Φ_m^2 and some fixed values of the normalized parameters B_s, B_o and B_m. From Fig. 4*a*, it is evident that the concentration of substrate decreases when Thiele modulus increases.

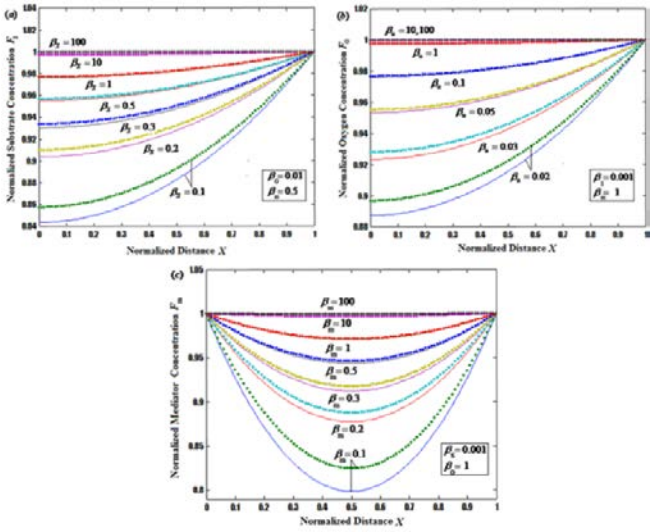

FIG. 3 A PLOT OF NORMALIZED CONCENTRATION PROFILES FOR (*a*) THE SUBSTRATE F_S, (*b*) THE OXYGEN F_O, AND (*c*) THE OXIDIZED MEDIATOR F_m. THE CURVES ARE PLOTTED USING EQS. 10 – 12 FOR VARIOUS VALUES OF THE REACTION CONSTANTS AND SOME FIXED VALUES OF PARAMETERS $\Phi_s^2 = \Phi_o^2 = \Phi_m^2 = 5$, $[S]_b = 0.01$ MMOL/L, $[O_2]_b = 0.001$ MMOL/L, AND $[M_{red}]_b = 0.05$ MMOL/L. SOLID LINES REPRESENT THE ANALYTICAL SOLUTION PRESENTED IN THIS WORK AND DOTTED LINES THE NUMERICAL SIMULATION

It is obvious that the substrate concentration F_s reaches the maximum value 1, at membrane /solution interface ($x = 1$). From Fig. 4*b*, it is evident that the value of oxygen concentration $F_o \approx 1$ for all small values of Φ_o^2 and large values of k_2 and k_3. Furthermore, there is a simultaneous decrease in the values of concentration of oxygen when the Thiele modulus increases. The concentrations of F_s and F_o have minimum values $1 - \Phi_s^2\left[2 + 2/(B_o + B_m) + 2/B_s\right]^{-1}$

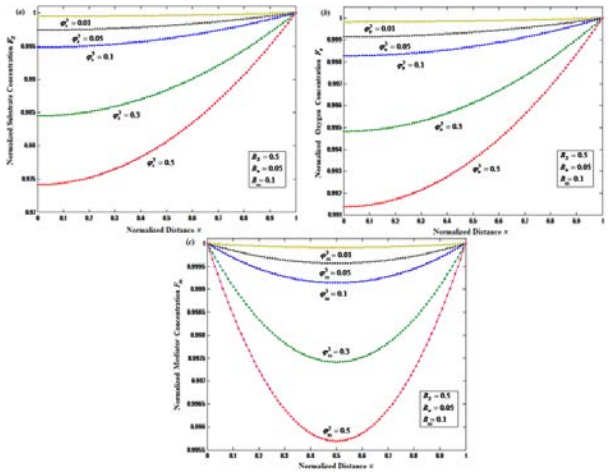

FIG. 4 NORMALIZED CONCENTRATION PROFILES FOR (*a*) THE SUBSTRATE F_S, (*b*) THE OXYGEN F_O, AND (*c*) THE OXIDIZED MEDIATOR F_m IN THE POLYMER MATRIX FOR VARIOUS VALUES OF THE THIELE MODULI Φ_s^2, Φ_o^2 and Φ_m^2 WHEN $B_s = 0.5$, $B_o = 0.05$ AND $B_m = 0.1$. SOLID LINES REPRESENT THE ANALYTICAL SOLUTION PRESENTED IN THIS WORK AND DOTTED LINES THE NUMERICAL SIMULATION

and $1 - \Phi_o^2 B_o \left[2 + 2/\left(B_o + B_m\right) + 2/B_s\right]^{-1} /\left(B_o + B_m\right)$ at $x = 0$. Upon careful evaluation of Fig. 4*c*, it is evident that there is a simultaneous decrease in the values of concentration of oxidized mediator when the Thiele modulus Φ_m^2 or thickness of the enzyme layer increases. The concentration F_m decreases initially and attains its minimum value $1 - \Phi_m^2 B_m \left[2 + 2/\left(B_o + B_m\right) + 2/B_s\right]^{-1} /\left(B_o + B_m\right)$ at x = ½ (middle of the membrane) and then increases. Upon comparison of analytical with the numerical, it is evident that both results are identical. Figure 5 is the

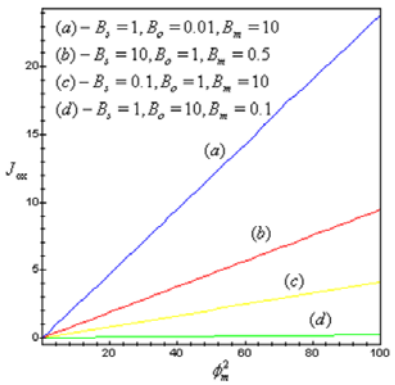

FIG. 5 THE NORMALIZED CURRENT J_{ox} VERSUS THE THIELE MODULUS Φ_m^2. THE CURRENT WERE COMPUTED USING EQ. 13 FOR VARIOUS VALUES OF THE NORMALIZED PARAMETERS (*a*) $B_s = 1, B_o = 0.01, B_m = 10$, (*b*) $B_s = 10, B_o = 1, B_m = 0.5$, (*c*) $B_s = 0.1, B_o = 1, B_m = 10$, AND (*d*) $B_s = 1, B_o = 10, B_m = 0.1$.

normalized current response J_{ox} for various values of the normalized parameters using Eq. 13. From Fig. 5, it is evident that the value of the current increases when Φ_m^2 increases. Fig. 6 indicates the profiles of

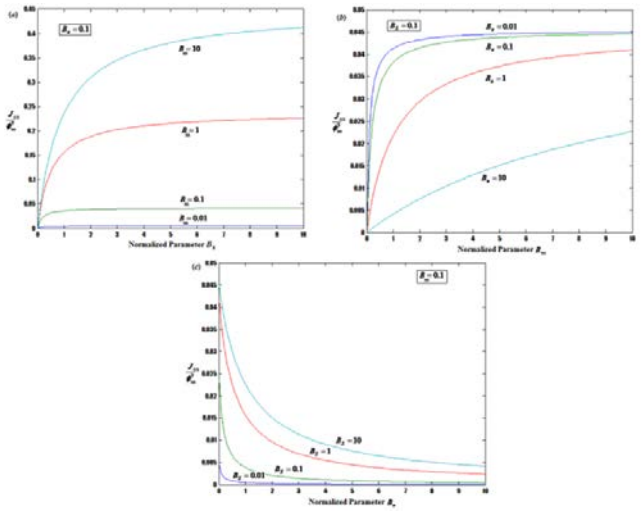

FIG. 6 PROFILES OF $\frac{J_{ox}}{\Phi_m^2}$ VERSES SURFACE CONCENTRATION OF (*a*) SUBSTRATE B_S, (*b*) OXIDIZED MEDIATOR B_m, AND (*c*) OXYGEN B_o IN THE POLYMER MATRIX. THE CURVES ARE COMPUTED USING EQ. 13 FOR VARIOUS VALUES OF THE NORMALIZED PARAMETERS B_m, B_O and B_S

J_{ox}/Φ_m^2 for various values of the normalized parameters B_s, B_o and B_m using Eq. 13. From Fig. 6*a*, it is clear that the value of the current increases when $\beta_m \left(= k_3/k_5\right)$ decreases. In addition, we noticed that the normalized current reaches the steady-state value when $B_m \le 0.1$ and $B_s \ge 4$. From Fig. 6*b*, it is obvious that the value of the current decreases when $\beta_o \left(= k_3/k_4\right)$ increases. Furthermore, the value of the current reaches the steady-state value when $B_o \le 0.01$ and $B_m \ge 4$. From Fig. 6*c*, it is observed that the value of the current increases when $\beta_s \left(= \left(k_2 + k_3\right)/k_1\right)$ decreases and reaches the steady-state value when $B_s \le 0.1$ and $B_o \ge 4$.

Tables 1-3 represent the comparison of analytical result with the numerical result of normalized concentration profiles for various values of the Thiele modulus. From the table 1, it is inferred that the concentration F_s decreases when Φ_s^2 increases or the thickness of the electrode increases. From the table 2, it is evident that the normalized substrate concentration $F_o \approx 1$ when the value of $\Phi_o^2 \le 1$. The comparison reveals that the relative difference between the analytical results and the numerical solution does not

exceed 3% for all values of parameters considered in the simulation.

Conclusions

We have analyzed the theoretical model for an immobilized oxidase enzyme electrode, previously described in Martens and Hall [14]. The system of non-linear steady-state reaction/diffusion equations of the model has been solved analytically. Approximate analytical expressions pertaining to the concentrations of species and current in three-substrate enzyme electrodes for all values of the Thiele moduli are obtained using the Homotopy perturbation method. Our results agree well with the numerical solution. This analytical result is useful for improve the sensors design. The extension of the procedure to the model with reduced mediator in the bulk solution seems possible. The theoretical results obtained can be used for the optimization of the thickness of the enzyme layer (or Thiele modulus) which produce significant change in both the magnitude of the current and the general behavior of the system.

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Appendix A

Analytical solution of the system of non-linear Eqs. 7a-7c using Homotopy perturbation method

In this appendix, we derive the solution of non-linear reaction/diffusion equations Eqs. 7*a*-7*c* using He's Homotopy perturbation method. To illustrate the basicconcepts of this method (HPM), we consider the following non-linear differential equation

$$L(u)+N(u)-f(r)=0\ , \qquad r\in\Omega \tag{A1}$$

where L is a linear operator, N is a nonlinear operator, u is an unknown function, and $f(r)$ is a given continuous function. We construct a homotopy which satisfies

$$(1-p)\left[L(u)\right]+p\left[L(u)+N(u)-f(r)\right]=0 \tag{A2}$$

Here $p\in[0,1]$ is an embedded parameter. Applying Eq. A2 for the Eqs. 7*a*-7*c*, we get

$$(1-p)\left(\frac{\mathrm{d}^2F_\mathrm{s}}{\mathrm{d}x^2}\right)+ p\left[\frac{\mathrm{d}^2F_\mathrm{s}}{\mathrm{d}x^2}-\Phi_s^2\left(1+\frac{1}{F_\mathrm{o}B_\mathrm{o}+F_\mathrm{m}B_\mathrm{m}}+\frac{1}{F_\mathrm{s}B_\mathrm{s}}\right)^{-1}\right]=0 \tag{A3}$$

$$(1-p)\left(\frac{\mathrm{d}^2F_\mathrm{o}}{\mathrm{d}x^2}\right)+ p\left[\frac{\mathrm{d}^2F_\mathrm{o}}{\mathrm{d}x^2}-\Phi_\mathrm{o}^2\left(1+\frac{1}{F_\mathrm{o}B_\mathrm{o}+F_\mathrm{m}B_m}+\frac{1}{F_\mathrm{s}B_\mathrm{s}}\right)^{-1}\frac{F_\mathrm{o}B_\mathrm{o}}{F_\mathrm{o}B_\mathrm{o}+F_\mathrm{m}B_\mathrm{m}}\right]=0 \tag{A4}$$

and

$$(1-p)\left(\frac{\mathrm{d}^2F_\mathrm{m}}{\mathrm{d}x^2}\right)+ p\left[\frac{\mathrm{d}^2F_\mathrm{m}}{\mathrm{d}x^2}-\Phi_\mathrm{o}^2\left(1+\frac{1}{F_\mathrm{o}B_\mathrm{o}+F_\mathrm{m}B_\mathrm{m}}+\frac{1}{F_\mathrm{s}B_\mathrm{s}}\right)^{-1}\frac{F_\mathrm{m}B_\mathrm{m}}{F_\mathrm{o}B_\mathrm{o}+F_\mathrm{m}B_\mathrm{m}}\right]=0 \tag{A5}$$

where linear operator $L(F_\mathrm{s})=d^2F_\mathrm{s}/dx^2$, $L(F_\mathrm{o})=d^2F_\mathrm{o}/dx^2$, and $L(F_\mathrm{m})=d^2F_\mathrm{m}/dx^2$ and non-linear operator

$$N(F_\mathrm{s})=\left(1+\frac{1}{F_\mathrm{o}B_\mathrm{o}+F_\mathrm{m}B_\mathrm{m}}+\frac{1}{F_\mathrm{s}B_\mathrm{s}}\right)^{-1},$$

$$N(F_\mathrm{o})=\left(1+\frac{1}{F_\mathrm{o}B_\mathrm{o}+F_\mathrm{m}B_\mathrm{m}}+\frac{1}{F_\mathrm{s}B_\mathrm{s}}\right)^{-1}\frac{F_\mathrm{o}B_\mathrm{o}}{F_\mathrm{o}B_\mathrm{o}+F_\mathrm{m}B_\mathrm{m}},$$

and

$$N(F_\mathrm{m})=\left(1+\frac{1}{F_\mathrm{o}B_\mathrm{o}+F_\mathrm{m}B_m}+\frac{1}{F_\mathrm{s}B_\mathrm{s}}\right)^{-1}\frac{F_\mathrm{m}B_\mathrm{m}}{F_\mathrm{o}B_\mathrm{o}+F_\mathrm{m}B_\mathrm{m}}.$$

Supposing the approximate solutions of Eqs. A3 – A5 have the form

$$\left.\begin{aligned}F_\mathrm{s}&=F_{\mathrm{s},0}+pF_{\mathrm{s},1}+p^2F_{\mathrm{s},2}+.....\\ F_\mathrm{o}&=F_{\mathrm{o},0}+pF_{\mathrm{o},1}+p^2F_{\mathrm{o},2}+.....\\ F_\mathrm{m}&=F_{\mathrm{m},0}+pF_{\mathrm{m},1}+p^2F_{\mathrm{m},2}+.....\end{aligned}\right\} \tag{A6}$$

Substituting Eq. A6 into Eqs. A3 – A5 (respectively), and equate the terms with the identical powers of p, we obtain

$$p^0 : \frac{\mathrm{d}^2 F_{s,0}}{\mathrm{d}x^2} = 0 \quad \text{(A7)}$$

$$p^1 : \frac{\mathrm{d}^2 F_{s,1}}{\mathrm{d}x^2} - \frac{\Phi_s^2}{1+1/\left(F_{o.0}B_o + F_{m,0}B_m\right)+F_{s,0}B_s} = 0 \quad \text{(A8)}$$

$$p^2 : \frac{\mathrm{d}^2 F_{s,2}}{\mathrm{d}x^2} - \Phi_s^2\left[\frac{F_{s,0}B_s\left(F_{o.0}B_o + F_{m,0}B_m\right)}{F_{o.0}B_o + F_{m,0}B_m + F_{s,0}F_{o.0}B_sB_o + F_{s,0}B_s + F_{s,0}F_{m,0}B_sB_m}\right] \frac{1}{1+1/\left(F_{o.0}B_o + F_{m,0}B_m\right)+F_{s,0}B_s}\left[\frac{F_{o.1}B_o + F_{m,1}B_m}{\left(F_{o.0}B_o + F_{m,0}B_m\right)^2} + \frac{F_{s,1}}{F_{s,0}^2 B_s}\right] = 0 \quad \text{(A9)}$$

and

$$p^0 : \frac{\mathrm{d}^2 F_{o,0}}{\mathrm{d}x^2} = 0 \quad \text{(A10)}$$

$$p^1 : \frac{\mathrm{d}^2 F_{o,1}}{\mathrm{d}x^2} - \frac{\Phi_o^2}{1+1/\left(F_{o,0}B_o + F_{m,0}B_s\right)+F_{s,0}B_s}\left[\frac{F_{o,0}B_o}{\left(F_{o,0}B_o + F_{m,,0}B_s\right)}\right] = 0 \quad \text{(A11)}$$

We can obtain the coefficient of p^2 for F_o as in the Eq. A9. Now substituting the third equation of A6 in A5, we can obtain

$$p^0 : \frac{\mathrm{d}^2 F_{m,0}}{\mathrm{d}x^2} = 0 \quad \text{(A12)}$$

$$p^1 : \frac{\mathrm{d}^2 F_{m,1}}{\mathrm{d}x^2} - \frac{\Phi_m^2}{1+1/\left(F_{o,0}B_o + F_{m,0}B_s\right)+F_{s,0}B_s}\left[\frac{F_{m,0}B_m}{\left(F_{o,0}B_o + F_{m,0}B_s\right)}\right] = 0 \quad \text{(A13)}$$

Similarly we can find the coefficient of p^2 for F_m as in the Eq. A9. The initial approximations (boundary conditions Eqs. 8a and 8b) are as follows:

$$F_{s,0}(x=1) = 1;\ \ F_{o,0}(x=1) = 1;\ \ F_{m,0}(x=1) = 1 \quad \text{(A14)}$$

$$F_{m,0}(x=0) = 1; \mathrm{d}F_{o,0}(x=0)\big/\mathrm{d}x = \mathrm{d}F_{s,0}(x=0)\big/\mathrm{d}x = 0 \quad \text{(A15)}$$

and

$$F_{s,i}(x=1) = 0;\ F_{o,i}(x=1) = 0;\ F_{m,i}(x=1) = 0;$$

$$i = 1,2,3,\ldots. \quad \text{(A16)}$$

$$F_{m,i}(x=0) = 0;\ \mathrm{d}F_{o,i}(x=0)/\mathrm{d}x = \mathrm{d}F_{s,i}(x=0)/\mathrm{d}x = 0;$$

$$i = 1,2,3,\ldots \quad \text{(A17)}$$

Solving the Eqs. A7, A10, and A12 and using the boundary conditions Eqs. A14 and A15, we get

$$F_{s,0} = 1 \quad \text{(A18)}$$

$$F_{o,0} = 1 \quad \text{(A19)}$$

and

$$F_{m,0} = 1 \quad \text{(A20)}$$

Substituting the above values of $F_{s,0}$, $F_{o,0}$ and $F_{m,0}$ in the Eq. A8, Eq. A11 and Eq. A13 and solving these equations, using the boundary conditions Eqs. A16 and A17, we can obtain the following results.

$$F_{s,1} = \frac{\Phi_s^2}{\left[2+2/\left(B_o + B_m\right)+2/B_s\right]}\left(x^2 - 1\right) \quad \text{(A21)}$$

$$F_{o,1} = \frac{\Phi_o^2}{\left[2+2/\left(B_o + B_m\right)+2/B_s\right]}\left[\frac{B_o}{B_o + B_m}\right]\left(x^2 - 1\right) \quad \text{(A22)}$$

and

$$F_{m,1} = \frac{\Phi_m^2}{\left[2+2/\left(B_o + B_m\right)+2/B_s\right]}\left[\frac{B_m}{B_o + B_m}\right]\left(x^2 - x\right) \quad \text{(A23)}$$

Adding Eq. A18 and A21, we get Eq. 10 in the text. Similarly we can get Eqs. 11 and 12 in the text. In Eq. A6, we can consider only two terms (two iterative steps). To find the next iterative, we can solve the Eq. A9, after substituting the Eqs. A21-A23. Accuracy of the solutions can be improved by considering next terms (ie by solving the Eq. A9).

Appendix B

Nomenclature

Symbols

$[E_t]$-Total active enzyme concentration in the matrix (mmol/L)

$[E_{ox}]$-Enzyme concentration of the oxidized mediator (mmol/L)

[ES]-Enzyme concentration of the substrate (mmol/L)

$[E_{red}]$-Reduced enzyme concentration (mmol/L)

$[O_2]$-Concentration of oxygen at any position in the enzyme layer (mmol/L)

$[O_2]_b$ -Oxygen concentration in the bulk electrolyte (mmol/L)

$[O_2]_\infty$ -Oxygen concentration in the bulk solution (mmol/L)

[S]-Concentration of substrate at any position in the enzyme layer (mmol/L)

$[S]_b$ -Substrate concentration in the bulk electrolyte (mmol/L)

$[S]_\infty$ -Substrate concentration in the bulk solution (mmol/L)

$[Med_{ox}]$ -Concentration of oxidised mediator at any position in the enzyme layer (mmol/L)

$[Med_{ox}]_b$ -Oxidised mediator concentration in the bulk electrolyte (mmol/L)

$[Med_{ox}]_\infty$ -Oxidised mediator concentration in the bulk solution (mmol/L)

D_o -Diffusion coefficient of oxygen ($cm^2 s^{-1}$)

D_s -Diffusion coefficient of substrate ($cm^2 s^{-1}$)

D_m - Diffusion coefficient of mediator ($cm^2 s^{-1}$)

d -Enzyme layer thickness (cm)

y -Distance from the electrode (cm)

k_1, k_4, k_5 - Rate constants (L $mol^{-1} s^{-1}$)

k_2, k_3 -Rate constants (s^{-1})

K_o -Partitioning coefficient for oxygen (none)

K_s - Partitioning coefficient for substrate (none)

K_m -Partitioning coefficient for mediator (none)

B_o -Normalized surface concentration of oxygen (none)

B_s - Normalized surface concentration of the substrate (none)

B_m - Normalized surface concentration of mediator (none)

F_o - Normalized oxygen concentration (none)

F_s - Normalized substrate concentration (none)

F_m - Normalized mediator concentration (none)

x - Normalized distance from the electrode (none)

J_{ox} - Normalized current (none)

Greek symbols

ϕ_o^2 - Thiele modulus for the oxygen (Normalized)

ϕ_s^2 - Thiele modulus for the substrate (Normalized)

ϕ_m^2 - Thiele modulus for the mediator (Normalized)

Subscripts

o - Oxygen

s - Substrate

m - Mediator

ox - Oxidized species

red - Reduced species

t - Total

∞ - Bulk solution

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TABLE. 1 COMPARISON OF NORMALIZED SUBSTRATE CONCENTRATION F_S WITH THE NUMERICAL RESULT FOR VARIOUS VALUES OF Φ_s^2 AND SOME FIXED VALUES OF NORMALIZED PARAMETERS $B_S = 0.04$, $B_O = 2.5$ AND $B_m = 0.2$.

x	$\Phi_s^2 = 0.1$			$\Phi_s^2 = 1$			$\Phi_s^2 = 5$			$\Phi_s^2 = 10$		
	Eq. 10	Numerical	% of deviation	Eq. 10	Numerical	% of deviation	Eq. 10	Numerical	% of deviation	Eq. 10	Numerical	% of deviation
0	0.9981	0.9981	0.00	0.9810	0.9813	0.03	0.9052	0.9118	0.72	0.8104	0.8352	2.97
0.2	0.9982	0.9982	0.00	0.9818	0.9821	0.03	0.9090	0.9153	0.69	0.8180	0.8416	2.80
0.4	0.9984	0.9984	0.00	0.9841	0.9843	0.02	0.9204	0.9257	0.57	0.8407	0.8609	2.35
0.6	0.9988	0.9988	0.00	0.9879	0.9880	0.01	0.9393	0.9433	0.42	0.8787	0.8934	1.65
0.8	0.9993	0.9993	0.00	0.9932	0.9933	0.01	0.9659	0.9679	0.21	0.9317	0.9395	0.83
1	1	1	0.00	1	1	0.00	1	1	0.00	1	1	0.00

TABLE. 2 COMPARISON OF NORMALIZED OXYGEN CONCENTRATION F_O WITH THE NUMERICAL RESULTS FOR VARIOUS VALUES OF Φ_o^2 AND SOME FIXED VALUES OF NORMALIZED PARAMETERS $B_S = 0.04$, $B_O = 2.5$ AND $B_m = 0.2$.

x	$\Phi_o^2 = 0.1$			$\Phi_o^2 = 1$			$\Phi_o^2 = 5$			$\Phi_o^2 = 10$		
	Eq. 10	Numerical	% of deviation	Eq. 10	Numerical	% of deviation	Eq. 10	Numerical	% of deviation	Eq. 10	Numerical	% of deviation
0	0.9982	0.9982	0.00	0.9824	0.9827	0.03	0.9122	0.9118	0.72	0.8490	0.8244	2.98
0.2	0.9983	0.9983	0.00	0.9831	0.9834	0.03	0.9157	0.9220	0.68	0.8548	0.8315	2.80
0.4	0.9985	0.9985	0.00	0.9853	0.9855	0.02	0.9263	0.9316	0.57	0.8725	0.8525	2.35
0.6	0.9989	0.9989	0.00	0.9888	0.9889	0.01	0.9438	0.9477	0.41	0.9022	0.8876	1.65
0.8	0.9994	0.9994	0.00	0.9937	0.9938	0.01	0.9684	0.9705	0.22	0.9445	0.9368	0.82
1	1	1	0.00	1	1	0.00	1	1	0.00	1	1	0.00

TABLE. 3 COMPARISON OF NORMALIZED MEDIATOR CONCENTRATION F_{m} WITH THE NUMERICAL RESULT FOR VARIOUS VALUES OF Φ_{m}^2 AND SOME FIXED VALUES OF NORMALIZED PARAMETERS $B_{\mathrm{s}} = 0.07$, $B_{\mathrm{o}} = 2.5$ AND $B_{\mathrm{m}} = 0.2$.

x	$\Phi_{\mathrm{m}}^2 = 0.1$			$\Phi_{\mathrm{m}}^2 = 1$			$\Phi_{\mathrm{m}}^2 = 100$			$\Phi_{\mathrm{m}}^2 = 300$		
	Eq. 10	Numerical	% of deviation	Eq. 10	Numerical	% of deviation	Eq. 10	Numerical	% of deviation	Eq. 10	Numerical	% of deviation
0	1	1	0.00	1	1	0.00	1	1	0.00	1	1	0.00
0.2	1	1	0.00	0.9998	0.9998	0.00	0.9775	0.9830	0.56	0.9326	0.9505	1.88
0.4	1	1	0.00	0.9997	0.9997	0.00	0.9663	0.9737	0.76	0.8998	0.9237	2.69
0.6	1	1	0.00	0.9997	0.9997	0.00	0.9663	0.9728	0.67	0.8989	0.9211	2.41
0.8	1	1	0.00	0.9998	0.9998	0.00	0.9775	0.9812	0.38	0.9326	0.9455	1.36
1	1	1	0.00	1	1	0.00	1	1	0.00	1	1	0.00

TABLE. 4 NUMERICAL VALUES OF THE PARAMETERS USED IN THIS WORK. THE FIXED VALUES OF THE PARAMETERS ARE $[S]_{\mathrm{b}} = 5\,\mathrm{mmol/L}$, $[\mathrm{O}_2]_{\mathrm{b}} = 1.2$ mmol/L, $[\mathrm{Med}_{\mathrm{red}}]_{\mathrm{b}} = 1$ mmol/L, AND $[E_{\mathrm{t}}] = 0.26$ mmol/L, $d = 1\times10^{-3}$ cm, $k_1 = 14{,}000$ $\mathrm{Lmol^{-1}s^{-1}}$, $k_2 = 0$ $\mathrm{Lmol^{-1}s^{-1}}$, $k_3 = 1{,}000\,\mathrm{Lmol^{-1}s^{-1}}$, $k_4 = 2\times10^{6}$ $\mathrm{Lmol^{-1}s^{-1}}$, $k_5 = 2\times10^{5}$ $\mathrm{Lmol^{-1}s^{-1}}$, $\beta_s = 0.0714$ MOL/L, $\beta_{\mathrm{o}} = 0.0005$ MOL/L, $\beta_{\mathrm{m}} = 0.005$ MOL/L. THESE PARAMETERS ARE USED IN MARTENS AND HALL [14].

Parameter	**Numerical values of parameter used in Martens and Hall [14]**	**Numerical values of parameters used in this work**			
		Fig-2(a)-(c)	**Fig-3(a)-(c)**	**Fig-4(a)-(c)**	**Table 1-3**
D_s **($\mathrm{cm^2s^{-1}}$)**	10^{-6}	Fig. 2 (a) $10^{-8.5}$ to 10^{-6}	10^{-7}	10^{-9} to 10^{-7}	Table (1) 10^{-9} to 10^{-7}
D_{o} **($\mathrm{cm^2s^{-1}}$)**	10^{-5}	Fig. 2 (b) $10^{-9.5}$ to 10^{-7}	10^{-6}	10^{-8} to 10^{-6}	Table (2) 10^{-9} to 10^{-7}
D_{m} **($\mathrm{cm^2s^{-1}}$)**	10^{-6}	Fig. 2 (c) 10^{-10} to 10^{-8}	10^{-7}	10^{-9} to 10^{-7}	Table (3) 10^{-11} to 10^{-7}
$B_s = [S]_{\mathrm{b}} / \beta_s$	0.07	0.50	Fig. 3 (a) 0.0001 to 0.1	0.50	0.07
$B_{\mathrm{o}} = [\mathrm{O}_2] / \beta_{\mathrm{o}}$	2.50	0.01	Fig. 3 (b) 0.00001 to 0.05	0.05	2.50
$B_{\mathrm{m}} = [\mathrm{Med}_{\mathrm{red}}]_{\mathrm{b}} / \beta_{\mathrm{m}}$	0.20	0.10	Fig. 3 (c) 0.0005 to 0.5	0.10	0.20
$\Phi_s^2 = \dfrac{d^2 k_3 [E_t]}{D_s [S]_{\mathrm{b}}}$	52	Fig. 2 (a) 0.01 to 3.16	5	Fig. 4 (a) 0.01 to 0.5	Table (1) 0.1 to 10
$\Phi_o^2 = \dfrac{d^2 k_3 [E_t]}{D_o [O_2]_{\mathrm{b}}}$	21.67	Fig. 2 (b) 0.1 to 31.60	5	Fig. 4 (b) 0.01 to 0.5	Table (2) 0.1 to 10
$\Phi_{\mathrm{m}}^2 = \dfrac{d^2 k_3 [E_t]}{D_{\mathrm{m}} [\mathrm{Med}_{\mathrm{red}}]_{\mathrm{b}}}$	260	Fig. 2 (c) 1 to 100	5	Fig. 4 (c) 0.01 to 0.5	Table (3) 0.1 to 300